Claims

1. A method for determination of amounts or relative proportions of more than one individual polynucleotide sequence or subgroups thereof in a polynucleotide mixture using a quantitative affinity aided solution hybridization in combination with fractionation for obtaining resolution, **characterized** in that the method comprises the consecutive steps of:
(a) providing, one or more organized pools with a preset optional number of soluble polynucleotide probes, each probe being complementary to an individual target polynucleotide sequence in the sample and being present in a surplus as compared to the target polynucleotides and each probe having approximately the same number of hybridizing nucleotides, which probes are made distinguishable by providing said polynucleotide probes with one or more resolution enabling tags, which change the size or mass and thereby provide the polynucleotide probes with different mobilities in the fractionation, separation or recording systems without disturbing the hybridization or capturing reaction;
(b) providing analyte polynucleotide sequences isolated from a sample comprising a mixture of target polynucleotide sequences with at least one affinity tag; and thereafter
(c) performing steps (i) and (ii) simultaneously, sequentially in the order (i) and (ii) or in the opposite order; wherein steps (i) and (ii) comprises
(i) allowing a hybridization reaction to take place between *a surplus* of soluble polynucleotide probes from step (a) and the analyte polynucleotide sequences from step (b) leading to a *quantitative* formation of soluble hybrids;
(ii) recovering quantitatively the hybrids, which have been quantitatively formed in step (i) by capturing said hybrids on a separation aiding tool provided with the affinity pair of the affinity tag of the analyte;
(d) quantitatively releasing the polynucleotide probes in an unmodified form from the hybrids captured to the separation aiding tool;
(e) recording the amount or relative proportions of distinguishable polynucleotide probes, the amount of which corresponds to the complementary polynucleotide sequences in the mixture of target polynucleotides in the sample.

2. The method according to claim 1, **characterized** in that for the determination of dynamic variations in the amounts or the relative proportions of polynucleotide transcripts or their subgroups in an individual organism, the soluble polynucleotide probes are designed from species or group-specific polynucleotide sequences hybridizing with selected more or less conserved or hypervariable regions from intragenomic

sequences specific for subgroups, species, subspecies of transcripts expressed in the organism.

3. The method according to claim 2, **characterized** in that the analyte polynucleotide sequences isolated from the sample comprising the mixed target population comprise messenger RNA (mRNA) or mRNA encoding genes (DNA).

4. The method according to claim 1, **characterized** in that for the determination of dynamic variations in the amounts or relative proportions of polynucleotide sequences representing individual organisms or subpopulations thereof in a target population, the soluble polynucleotide probes are designed from species or group-specific polynucleotide sequences hybridizing with selected more or less conserved or hypervariable region from intragenomic sequences specific for and/or representing different phylogenetic levels allowing the identification of subgroups, species, subspecies within the mixed target population.

5. The method according to claim 4, **characterized** in that the polynucleotide analytes isolated from the sample comprising the mixed target population comprise ribosomal RNA or DNA.

6. The method according to claim 1, **characterized** in that the resolution enabling tag may simultaneously act as a tracer, affinity and/or primer tag.

7. The method according to claim 4, **characterized** in that the resolution enabling tag is selected from a group consisting of polynucleotide residues, amino acid residues, peptides, sugar residues, haptens and ligands.

8. The method according to claim 4, **characterized** in that the resolution enabling tag, which additionally may act as an affinity and/or primer tag is a oligonucleotide residue.

9. The method according to claim 4, **characterized** in that the resolution enabling tag, which additionally may act as an affinity tag and/or a tracer tag is an amino acid sequence or a peptide.

10. The method according to claim 4, **characterized** in that the resolution enabling tag, which additionally may act as a tracer tag is selected from a group consisting of labels recordable by fluorescence, luminescence, infrared absorption, electromagnetic properties, radioactivity and enzymatic activity.

11. The method according to claim 1, **characterized** in that the preset optional number of soluble polynucleotide probes in the pool is more than one preferably more than five, most preferably more than ten.

12. The method according to claim 1, **characterized** in that the amount of the individual, quantitatively captured and released polynucleotide probes is recorded with a fully or partly automized recording system, which is selected based on the applied resolution enabling tags.

13. The method according to claim 12, **characterized** in that the recording system is selected based on resolution enabling tags and comprises mass spectrometry, electrophoretic or chromatographic techniques.

14. The method according to any of claim 1, **characterized** in that the amount of the quantitatively recovered primer tagged probes are released and subsequently amplified and optionally tracer tagged before, during or after the PCR-reaction and thereafter recorded with a recording system selected based on the resolution enabling tags.

15. The method according to claim 1, **characterized** in that the polynucleotide probes are DNA fragments, synthetic or recombinant polynucleotide sequences or modified polynucleotide sequences.

16. The method according to claim 1, **characterized** in that a comparative, quantitative assessment of variations in the amounts of individual polynucleotide sequences or organisms and subgroups thereof in a population or mixture of polynucleotide sequences by providing a set of multiple test kits, at least one test kit for each sample to be compared, each of said test kit comprising organized pools with identical soluble polynucleotide.

17. The method according to claim 16, **characterized** in that the individual test kits, wherein the resolution enabling tag is not a tracer tag, a set of multiple test kits is provided with tracer tags each being distinguishable from the other by the emitted signal.

18. The method according to claim 1, **characterized** in that the analyte polynucleotide sequences provided with an affinity tag are captured on the solid support before contacting it with the probe pools.

19. A test kit for carrying out the method according to any of claims 1-18, **characterized** in that the test kit comprises one or more organized pools, with a preset optional number of soluble polynucleotide probes, each probe being complementary to an individual target polynucleotide sequence in the sample and being present in a surplus as compared to the target polynucleotides in the samples, each having approximately the same number of hybridizing nucleotides, which probes are made distinguishable by providing each polynucleotide probes with one or more resolution enabling tags, which change the size or mass and thereby provide the polynucleotide probes with different mobilities in the fractionation, separation or recording systems without disturbing the hybridization or capturing reaction, each pool of polynucleotides probes being placed in an organized manner in their own vessels, which are separate or joined together.

20. The test kit according to claim 19, **characterized** in that for the determination of dynamic variations in the amounts or relative proportions of polynucleotide transcripts or their subgroups in an individual organism, the soluble polynucleotide probes are designed from species or group-specific polynucleotide sequences hybridizing with selected more or less conserved or hypervariable region from intragenomic sequences specific for subgroups, species, subspecies of transcripts expressed in the organism.

21. The test kit according to claim 20, **characterized** in that the polynucleotide analytes isolated from the sample comprising the mixed target population comprise messenger RNA (mRNA) or mRNA encoding genes (DNA).

22. The test kit according to claim 19, **characterized** in that for the determination of dynamic variations in the amounts or relative proportions of polynucleotide sequences representing individual organisms or subpopulations thereof in a target population, the soluble polynucleotide probes are designed from species or group-specific polynucleotide sequences hybridizing with selected more or less conserved or hypervariable region from intragenomic sequences specific for and/or representing different phylogenetic levels allowing the identification of subgroups, species, subspecies within the mixed target population.

23. The test kit according to claim 22, **characterized** in that the polynucleotide analytes isolated from the sample comprising the mixed target population comprise ribosomal RNA or DNA.

24. The test kit according to claim 19, **characterized** in that the resolution enabling tag may simultaneously act as a tracer, affinity or primer tag.

25. The test kit according to claim 24, **characterized** in that the resolution enabling tag is selected from a group consisting of polynucleotide residues, amino acid residues, peptides, sugar residues, haptens and ligands.

26. The test kit according to claim 24, **characterized** in that the resolution enabling tag, which additionally may act as an affinity tag and/or primer tag is an oligonucleotide residue.

27. The test kit according to claim 24, **characterized** in that the resolution enabling tag, which additionally may act as an affinity tag and/or a tracer tag is an amino acid sequence or a peptide.

28. The test kit according to claim 24, **characterized** in that the resolution enabling tag, which additionally may act as a tracer tag is selected from a group consisting of labels recordable by fluorescence, luminescence, infrared absorption, electromagnetic properties, radioactivity and enzymatic activity.

29. The test kit according to claim 24, **characterized** in that the preset optional number of soluble polynucleotide probes in the pool is more than one preferably more than five, most preferably more than ten.

30. The test kit according to claim 24, **characterized** in that the soluble pools of polynucleotide probes are placed in wells on a microtiter plate.

31. The test kit according to claim 19, **characterized** in that the polynucleotide probes are DNA fragments, synthetic, recombinant or modified polynucleotide sequences.

32. The test kit according to claim 19, **characterized** in that for a comparative, quantitative assessment of variations in the amounts or relative proportions of individual target polynucleotide sequences or organisms and subgroups thereof in a population or mixture of polynucleotide sequences comprises a set of test kits, wherein at least one identical test kit having identical pools of polynucleotide probes for each sample to be compared.

33. The test kit according to claim 32, **characterized** in that each individual test kits in the set of multiple test kits is provided with tracer tags, which are distinguishable from each other by the emitted signal.

34. Use of the test kit according to claim 19 for determining variations in the amounts or relative proportions of more than one target polynucleotide sequence in a mixture with the method according to claim 1 for assessing hygienic conditions and epidemiologic situations, effects of external stimuli or treatment modalities on a microbial population.

35. The use according to claim 34, wherein the external stimulus or treatment is selected from a group consisting of treatment with antibiotics or hygienic measures.

36. Use of the method according to claim 1 for determining variations in the amount of more than one polynucleotide sequence in a mixture with the method according to claim 1 for assessing hygienic conditions and epidemiologic situations, effects of external stimuli or treatment modalities on a microbial population.

37. The use according to claim 36, wherein the external stimulus or treatment is selected from a group consisting of treatment with antibiotics or hygienic measures.